Low pH and an Intracellular Environ Induce Staphylococcus epidermidis Small Colony Variant Formation

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Abstract
Small colony variants (SCVs) of S. epidermidis are naturally occurring, slow-growing subpopulations of bacteria that emerge under conditions of environmental stress and may persist intracellularly. The growth of SCVs may be limited by reduced metabolic activity and low pH in lysosomes or phagosomes, which may induce S. epidermidis SCV formation. We determined whether neutralizing pH in lysosomes would induce S. epidermidis SCV formation compared to normal pH. We also assessed the effect of low pH and intracellular growth on the selection of S. epidermidis SCVs.

Methods
We grew S. epidermidis strains RP62A and two S. epidermidis prosthetic joint infection isolates, IDRL 8864, and IDRL 8933, were studied. Bacteria were grown in Eagle’s Minimum Essential Medium (EMEM) supplemented with 1% fetal bovine serum (FBS), pH 7.4. To decrease pH, EMEM was adjusted to pH 4, 5.5, and 6.5. To increase pH, EMEM was adjusted to pH 7.4 in the presence of 50 mM HEPES. In addition, human lung fibroblasts were grown in 10% FBS medium with buffers containing Na2HPO4 and tartaric acid to achieve pH values of 4, 5.5, and 6.5. Following 30 minutes of incubation, pH was adjusted to pH 7.4 and bacterial growth was assessed by colony counts on days 0, 3, and 5. Colony counts were performed on days 0, 3, and 5. Colony counts were adjusted for log10 CFU/mL.

Figure 1: Low pH induces SCV formation

Conclusions
Small colony variants (SCVs) are naturally occurring, slow-growing subpopulations of bacteria that emerge in response to diverse environmental pressures and may persist intracellularly. The growth of SCVs may be limited by reduced metabolic activity and low pH in lysosomes or phagosomes, which may induce S. epidermidis SCV formation. We determined whether neutralizing pH in lysosomes would induce S. epidermidis SCV formation compared to normal pH. We also assessed the effect of low pH and intracellular growth on the selection of S. epidermidis SCVs.

Figure 2: Colony phenotype

Conclusions and Discussion
Low pH and an intracellular environ promote the formation of SCVs. Acidic environments, such as within lysosomes or phagosomes, may induce S. epidermidis SCV formation. It has also been shown that S. epidermidis SCVs may revert back to fast growth, in vitro. Although unknown in vivo, their intracellular persistence and SCV formation may be a strategy to survive within the intracellular environment. Low pH conditions and SCV formation have been shown to be triggered by antibiotics. These observations align with our findings emphasizing how SCVs may recur if re-exposed to promote SCV formation.

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Future Directions
- Evaluate whether acidic pH variants or factors induce SCVs.
- Determine whether neutralizing pH in lysosomes would decrease the frequency of SCVs.
- Determine if S. epidermidis escapes the phagosome.
- Evaluate intracellular persistence of S. epidermidis in human osteoblasts, macrophages, and neutrophils.

Figure 3: Frequency of SCVs overtime during intracellular infection

Figure 4: Localization of Intracellular S. epidermidis

References
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